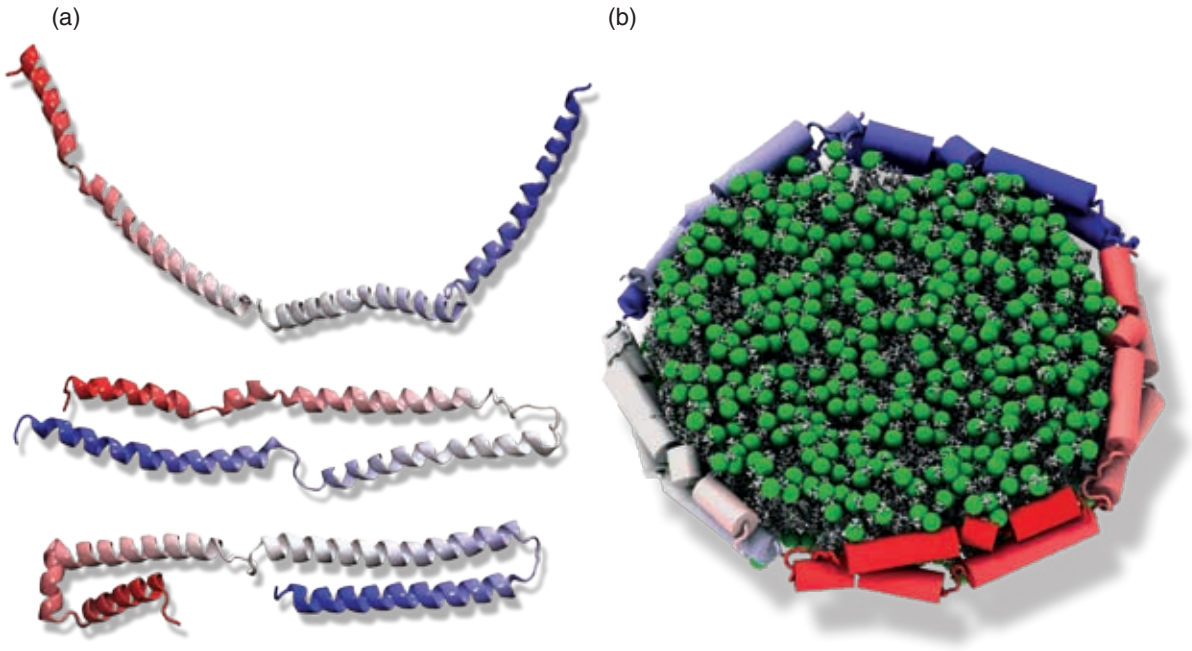
Simulating the Biomolecular Structure of Nanometer-Size Particles

HUMAN disease caused by pathogens such as viruses and bacteria often results in infection. Infectious diseases may, in turn, affect human proteins and alter cellular function. Although researchers are still learning how those changes occur, the pharmaceutical industry often develops drugs to treat diseases by observing cell behavior. Nearly 60 percent of current drug molecules target proteins on the surface of cell membranes and partition the membrane's intracellular components from its extracellular environment.

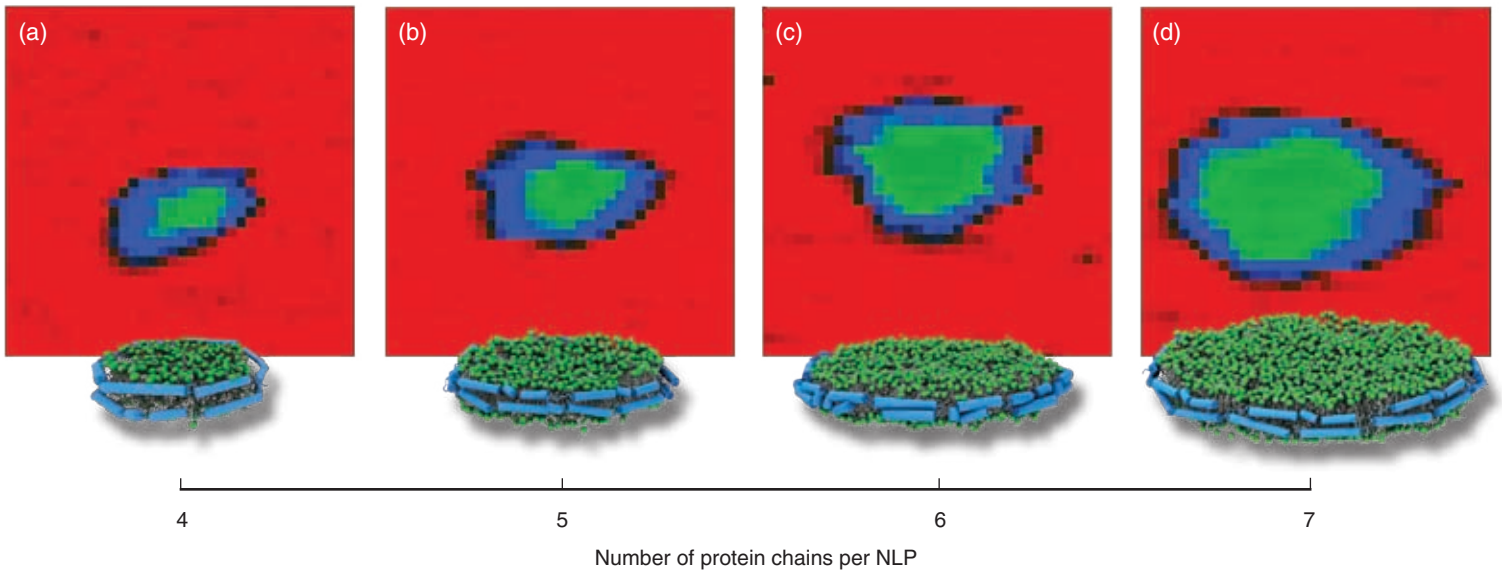
Membrane proteins are involved in an array of cellular processes required for organisms to survive, including energy production, communication between cells, and drug interactions. "Membrane proteins are the first responders or mediators for what passes through every cell in our body," says Livermore chemist Paul Hoeprich, who works in the Chemistry, Materials, Earth, and Life Sciences (CMELS) Directorate. "They connect the outside world to the inside cellular world."

Membrane proteins are exceptionally difficult to study partly because they are insoluble and tend to aggregate or precipitate when removed from their natural environment. "A change in environment will alter the structure of a membrane protein to such an extent that it becomes nonfunctioning," says computational chemist Richard Law, also in the CMELS Directorate. "In addition, these proteins are hydrophobic. To hide from water, they stick together and form a 'blob,' making it impossible to figure out how they function."

Because membrane proteins are insoluble, their structures cannot be easily mapped by x-ray crystallography, a technique commonly used to examine protein structures. Of the more than 45,000 protein structures known today, less than 1 percent are membrane proteins. To capture these proteins, scientists are constructing nanolipoprotein particles (NLPs) in the laboratory and using them as surrogates for cell membranes. NLPs are similar to the high- and low-density lipoprotein particles in the bloodstream—the "good" and "bad" cholesterol that moves fats and lipids through our bloodstream—and they mimic the membrane protein's natural cellular environment. Because NLPs are smaller and more stable in aqueous environments than the hydrophobic cell membranes, they offer an excellent platform for studying the structure and function of membrane proteins.



(a) Computer simulations show chains of the apolipoprotein E4-22K, where colors denote numbering from one end of a molecule (blue) to the other (red). (b) When chains link together around a group of lipids (green), they form a nanolipoprotein particle (NLP). Here, red, white, and blue denote individual folded proteins. An animated NLP is available online at www.llnl.gov/str/JulAug08/videos/nanolipoprotein.swf.



Atomic force microscopy images show that the number of protein chains surrounding a lipid determines the size of an NLP. Research indicates that NLPs occur in four sizes: (a) 14.5, (b) 19.0, (c) 23.5, and (d) 28.0 nanometers in diameter. (Micrographs taken by Craig Blanchette.)

A "Grand" Visualization

With funding from Livermore's Laboratory Directed Research and Development (LDRD) Program, Hoeprich and Law are combining their expertise in experimental and computational science to model the structure of NLPs at the nanometer scale and examine protein behavior in detail. Law's simulation effort is one of 17 projects selected for Livermore's second annual Computing Grand Challenge Program. Each year, the Grand Challenge Program allocates time on Livermore's high-performance computers to projects that are vital to the Laboratory's missions, allowing researchers to simulate processes ranging from earthquakes to plutonium decay.

"Our ultimate goal is to analyze the structure and function of membrane proteins," says Law. "First, we must understand the structure of a nanolipoprotein particle, which we will use to capture the membrane proteins involved in host–pathogen interactions." Law's simulations allow Hoeprich's team to characterize the biomolecular structure of a protein called apolipoprotein and examine how it combines with lipids to create NLPs. Results from these simulations will also be used to design laboratory experiments with the Linac Coherent Light Source at the Stanford Linear Accelerator Center. (See the box on p. 22.)

High-Tech Tools

The NLP simulations were based on data acquired in laboratory experiments. Using high-resolution techniques such as atomic force microscopy, ion mobility spectroscopy, and electron microscopy, Hoeprich and his team imaged thousands of the NLPs they assembled in the laboratory. In analyzing data from past experiments, they found that stable NLPs exist in four sizes—at 14.5, 19.0, 23.5, and 28.0 nanometers in diameter. Prior to this finding, researchers did not realize that NLPs could be quantized and would follow rules more closely associated with physics and physical chemistry. According to Hoeprich, the different imaging methods gave remarkably similar results about NLP structure, which provided a starting point for the simulations.

For the Grand Challenge project, Law developed calculations to run on the Thunder and Zeus supercomputers, two of the Laboratory's Linux clusters. Using experimental data from Hoeprich's team, Law modeled the structure of an apolipoprotein called E4-22K. He then simulated E4-22K apolipoproteins wrapping around a group of lipids to form the NLPs created in the laboratory.

Each NLP contains millions of atoms. Because atoms move so quickly, Thunder must take "snapshots" of a simulated NLP about every 2 femtoseconds (2 quadrillionths of a second, or 2×10^{-15} seconds) to accurately capture each atom's position. After a few weeks of computer calculations, Thunder produced millions of snapshots, which were then combined to form continuous simulations, each 20 to 100 nanoseconds long.

Law's high-resolution models corroborated the experimental results that E4-22K NLP structures occur in four distinct sizes. In addition, the simulations indicated that a particle's size is related to the number of apolipoproteins surrounding the lipids. With E4-22K, four to seven protein chains surround the lipids.

The Linac Coherent Light Source

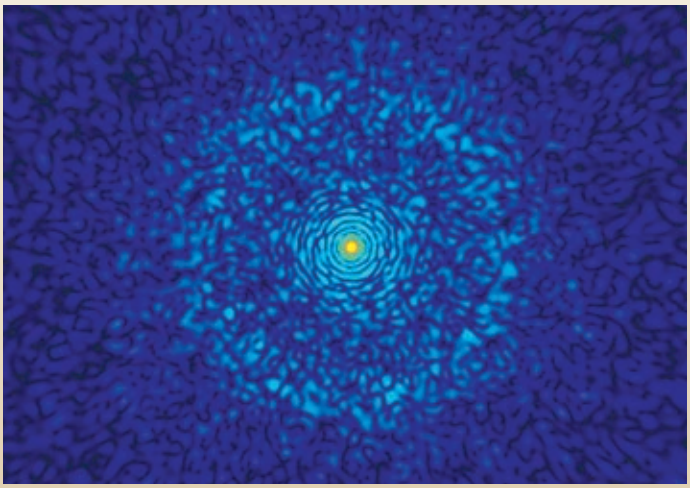
Although their methods are somewhat different, Livermore scientists Richard Law and Brian Bennion share a common "grand challenge" goal—to determine the structure and function of nanolipoprotein particles (NLPs) and membrane proteins. Using data from Law's simulations, Bennion is modeling NLPs in very low-pressure environments to predict the behavior of a particle exposed to the x-ray beam of the Linac Coherent Light Source (LCLS).

LCLS is under construction at the Stanford Linear Accelerator Center and will come online in 2009. In the meantime, Bennion's simulations are helping scientists plan for future LCLS experiments. His first set of simulations illustrates how the vacuum conditions required for the LCLS beam might influence the movement of NLP atoms. This information will be incorporated into a second set of simulations to determine how the vacuum conditions might affect NLP components such as water, proteins, and lipids.

In the LCLS experiments, an injector machine will spray tiny droplets of water mixed with NLPs through the vacuum chamber to the machine's interaction region. Ideally, each water droplet will contain only one NLP. Inside the vacuum, an x-ray beam will strike the NLP–water droplet, and a camera will record the diffraction pattern as the x rays interact with the atoms of the NLP. By piecing together the recorded images, researchers can create a three-dimensional view of the particle.

Preliminary simulations indicate that if the shell of water surrounding an NLP is not uniformly thick, the diffraction pattern may be blurred because of uneven exposure to the x-ray beam. To determine if this prediction is accurate, Bennion plans to calculate thousands of diffraction patterns for the same NLP.

"It takes 12 hours of computing time to calculate a 1-picosecond molecular dynamics simulation of an NLP in vacuum conditions," says Bennion. "We'd like to have at least 500 picoseconds total. That's about a year's worth of simulation time on a supercomputer running 24 hours a day." With only about 25 picoseconds of data to date, the computer has a lot more work to do.



This snapshot from a Livermore simulation predicts the diffraction pattern that will occur when the Linac Coherent Light Source shoots an x-ray beam at a nanolipoprotein particle (NLP). Atomic bonds appear in the pattern's outer rings. The yellow–red spot in the middle indicates where 95 percent of the beam traveled through the NLP to the machine's beam stop.

"Law's simulations have been extremely helpful because they allow us to visualize an NLP," says Hoeprich. "Before this Grand Challenge project, no one had simulated the nanoparticles in so much detail."

A Closer Look at Cell Behavior

Future simulations will focus on the process in which an intact membrane protein is implanted inside an NLP environment. "Now that we can determine the type and size of NLPs we've created," says Law, "we can match them with the appropriate membrane proteins to examine the insertion process." These simulations will help scientists determine whether the NLP environment is as similar to the membrane proteins' natural environment as they believe it to be. This modeling effort will also advance research to determine how drugs interact with membrane protein receptors and the cells they affect—knowledge that is critical for developing potential treatments for diseases.

In addition, a better understanding of how membrane proteins assemble and function will help researchers evaluate cellular response to chemical and biological warfare agents. With such information, they can improve countermeasures and approaches for detecting and minimizing the threat of exposure to these agents. "Knowing a protein's structure is important in understanding its function," says Law, "and, in the case of disease, designing drugs to alter that behavior."

—Kristen Light

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